Life Sciences, Vol. 28, pp. 1975-1986
Printed in the U.S.A.

Pergamon Press

MINIREVIEW

THE MELATONIN RHYTHM GENERATING SYSTEM: DEVELOPMENTAL ASPECTS

D.C. Klein, M.A.A. Namboodiri, and D. A. Auerbach

Section on Neuroendocrinology, Laboratory of Developmental Neurobiology
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, MD 20205

Summary

Development of the melatonin rhythm generating system, including the suprachiasmatic nucleus, sympathetic innervation, and biochemistry of the pineal gland is reviewed. This system offers investigators an interesting opportunity to study the effects of drugs, hormones, and other factors on the developmental appearance of specific structures and biochemical parameters, and to determine the role, if any, each has in the development of an integrated neural system.

This review deals with the development a mammalian regulatory system which generates the circadian rhythm in melatonin (Fig. 1) (1). Some preliminary comments on melatonin may be of interest, however, before discussing this system.

Melatonin is probably the hormone mediating the only well documented physiological action of the pineal gland, a photic-induced antigonadotrophic effect seen best in the hamster (2,3). In these seasonal breeders, gonadal regression occurs when there is a shift in the environmental lighting schedule from L:D 14:10 to L:D 10:14. Pinealectomy prevents this regression. Attempts to understand the mechanism by which this occurs has led to the discovery and description of a complex regulatory system which functions in mammals to generate a precise circadian rhythm in melatonin production (1,2) (Fig. 1). More recently the exciting observation has been made that the antigonadal effects of exogenously administered melatonin are manifest only during discrete periods of the day (5,6). We suspect that gonadal regression occurs when peaks in melatonin production and melatonin sensitivity converge. The phase relationship of these two rhythms is probably controlled by environmental lighting acting through the regulatory systems which control rhythms in melatonin sensitivity and melatonin production (4).

Although the system generating the rhythm in melatonin production has been reviewed frequently (1,2,3,7), little attention has been given to a large body of work on its ontogeny, reviewed here. This should be of specific interest to students of the pineal gland, and of general interest to investigators studying development of the nervous system.

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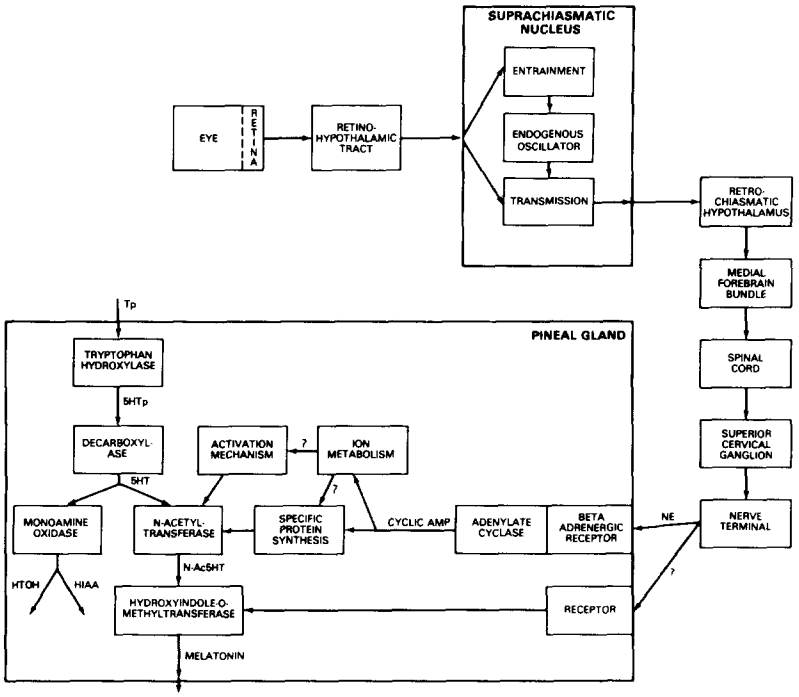

Fig. 1

The melatonin rhythm generating system. A schematic representation of the system which generates the circadian rhythm in melatonin. NE, norepinephrine; cyclic AMP, adenosine 3',5'-cyclic monophosphate; Tp, tryptophan; 5HTp, 5-hydroxytryptophan; 5-HT, 5-hydroxytryptamine, serotonin; HIAA, 5-hydroxyindole acetic acid; HTOH, 5-hydroxytryptophol; N-Ac5-HT, N-acetylserotonin. The question marks indicate unproven hypotheses. From (8).

Development of the melatonin rhythm generating system has been studied primarily in the rat. Accordingly, the material cited here refers to this species, unless otherwise indicated. It should be noted, however, that a rhythm in the melatonin content of the pineal gland, blood, CSF and urine has been found in all mammals examined (2,8). In addition, regardless of life style, high values always occur during the dark period of a 24 hour lighting cycle.

I. Characteristics of The Melatonin Rhythm Generating System

There are two major components of the melatonin rhythm generating system the suprachiasmatic nuclei (SCN) of the hypothalamus and the pineal gland (Fig. 1). The SCN appear to function as a self-sustaining endogenous biological clock (9,10,11), which, among other effects, stimulates the pineal gland every night. Signals reach the gland via a circuit passing through both central and peripheral neural structures (7). The SCN receive photic input from the eyes via a retinohypothalamic projection (12). Light has two effects: it resets

the clock mechanism so that it is precisely synchronized with the environmental lighting schedule and it blocks transmission of stimulatory signals to the pineal gland (13).

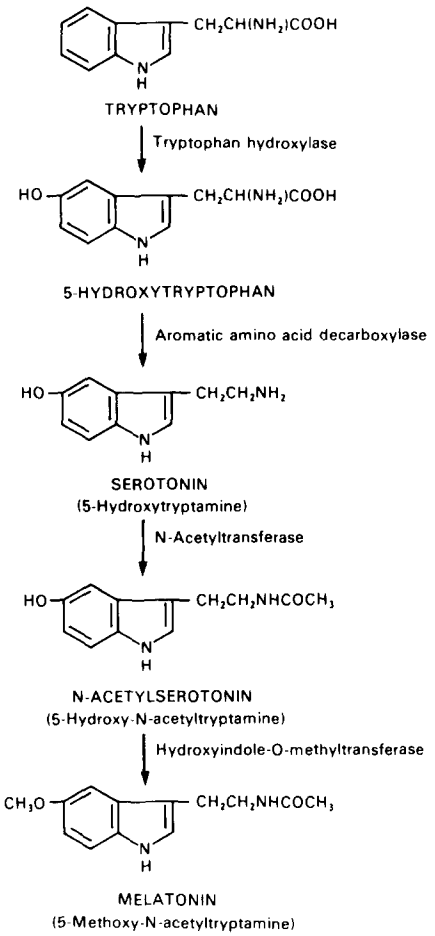

Fig. 2

The converstion of tryptophan to melatonin.

The pineal gland converts tryptophan to melatonin (Fig. 2) and contains a regulatory system (Fig. 1) which mediates neural control of large daily changes in melatonin production (Fig. 3) (14). The regulatory mechanism involves a membrane bound adrenergic-cyclic AMP receptor-transducer system. Cyclic AMP stimulates melatonin production by increasing the activity of serotonin N-acetyltransferase, thereby enhancing the production of N-acetylserotonin, which then increases the production of melatonin (N-acetyl-5-methoxytryptamine)

through a mass action effect. The increase in serotonin N-acetyltransferase activity concomittantly decreases pineal serotonin.

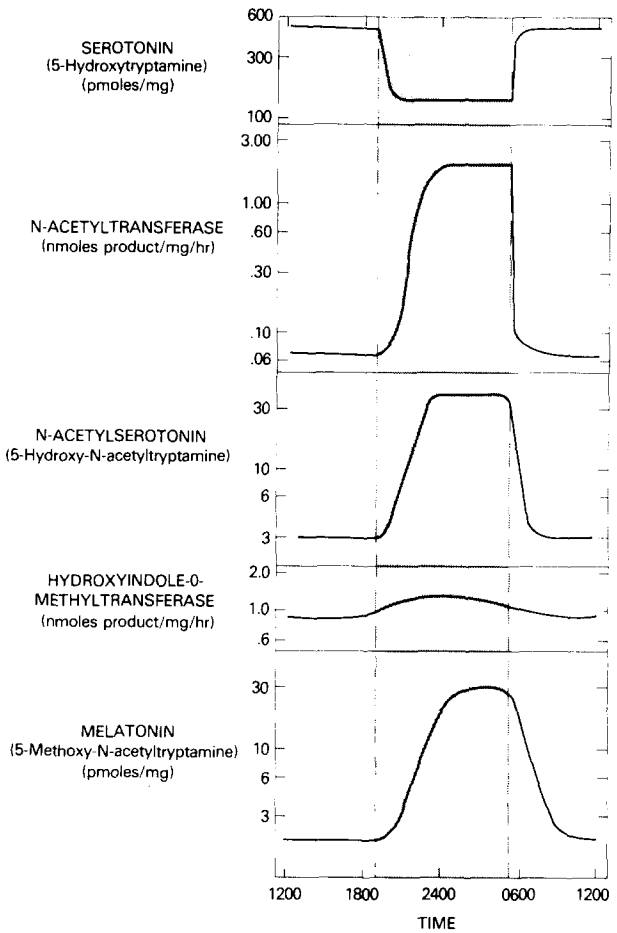

Fig. 3

Rhythms in indole metabolism in the rat pineal gland. The daily variations in the concentration of metabolites and activities of enzymes have been abstracted from reports in the literature. From (8).

II. Development of the Melatonin Rhythm Generating System (Fig. 4)

A. The SCN and Retinohypothalamic Projection:

The neurons of the rat SCN appear to originate during a 4 day period which starts on the 14th day of gestation (15,16,17). It seems from studies

in the mouse that the optic tract or the eye itself influences the development of the SCN, because in genetically anophthalmic mice the gross size of one or both SCN are reduced, the number of neurons is less than normal and some dendrites of SCN neurons fail to fully develop (18).

A distinct metabolic change in the rat SCN occurs one day prior to birth as indicated by a marked increase in uptake of 2-deoxyglucose (19). A second metabolic change occurs on the first day of life, when a distinct day/night difference in 2-deoxyglucose uptake becomes clearly detectable. This day/night difference in uptake is not seen in other areas of the brain, and is likely a reflection of metabolic activity associated with the self-sustaining clock mechanism.

Although the neurons of the SCN appear to be formed and metabolically functional soon after birth, significant maturation of the SCN neuropil occurs during the postnatal period. A variety of synaptic types begin to appear on day 4 and substantial maturation occurs during days 4 to 8 (20).

The retinohypothalamic projection to the SCN is first detectable during days 3 to 4 and progressivly develops over the next two weeks (21,22). However, the stimulatory effect of light on 2-deoxyglucose uptake seen in the adult can be observed in the neonatal animal before the retinohypothalamic projection invades the SCN (19).

This latter set of observations is somewhat troublesome because it is generally assumed that the retinohypothalamic projection mediates all the effects of light on the SCN in the adult. Among possible explanations are the existance of an alternative visual pathways to the SCN from the eye, mechanisms allowing for non-retinal photoreception influencing the SCN, and the possibility that elements of the retinohypothalamic projection are present and functional prior to 3 days of age but are not detectable by the techniques used. It is also possible that light-related changes in locomotor activity might influence the metabolism of the SCN.

As indicated above the neural circuit between the SCN and the pineal gland includes both central and peripheral components. Little is known about the precise brain structures involved. However, the neural circuit passes through the retrochiasmatic area of the hypothalamus, the medial forebrain bundle, the brainstem reticular formation and the intermediolateral cell column of the spinal cord. It exits the spinal cord in preganglionic fibers projecting to the superior cervical ganglia (7). Neurons in the superior cervical ganglia send projections to the pineal gland; the development of sympathetic innervation of the pineal gland is discussed below. It should be noted that the daily rhythm in pineal N-acetyltransferase is first detectable 4 days after birth (23), indicating that the circuit between the SCN and pineal gland is functional at this time, although immature.

As pointed out above, one effect of light is to synchronize endogenous rhythmic activity with the environmental lighting cycle. Clearly, light is the major synchronizing or entraining cue for the adult. However, evidence has been presented which indicates that maternal cues may provide an entraining function to the fetus, and to neonatal animals maintained in constant darkness (24,25).

DEVELOPMENTAL SCHEDULE OF THE MELATONIN RHYTHM GENERATING SYSTEM IN THE LABORATORY RAT

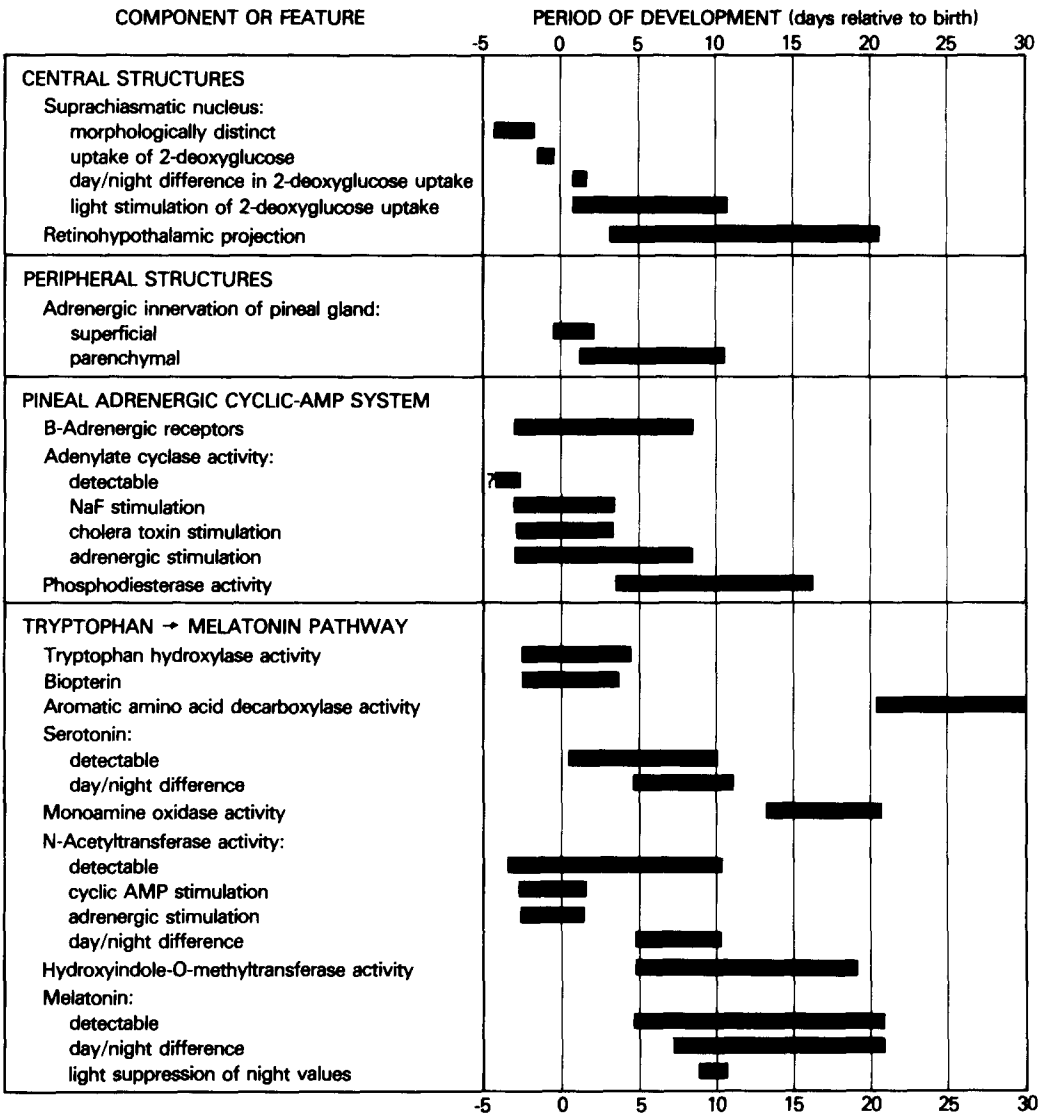

Fig. 4

Developmental schedule of the melatonin rhythm generating system in the rat. In most cases the designated period of development is the time during which the component or characteristic indicated occurs or changes substantially and approximates adult values. The levels of enzymes or metabolites are evaluated on a per mg protein basis. Accordingly, as the pineal gland increases in size the total activity of an enzyme might increase, although the specific activity does not. This increase is not regarded here as a developmental increase. The designation of developmental period in a somewhat subjective judgement; the original references should be referred to in order to obtain a more accurate description of the development of a specific component or characteristic of this system.

It is apparent that in the adult all the effects of light on the pineal gland are mediated by the eye (1). However, some evidence has appeared which suggests that light might act on the pineal gland of the neonatal rat via non-retinal mechanisms (26). It should be added that due to the design of the original experiments, it is difficult to separate the effects of light-deprivation from more nonspecific effects of stress associated with the procedure used to deprive the pups of light in those experiments, i.e. hooding. It appears that stress can result in adrenergic stimulation of the pineal gland, especially in neonatal animals (27).

B. Sympathetic Innervation

Innervation of the pineal gland by post-ganglionic neurons from the superior cervical ganglia is seen at one day of age as catecholamine-containing fibers on the surface of the gland (28). Within the next few days there is marked and extensive invasion of the gland by these fibers, followed by progressive maturation. Before the end of the second week of life innervation exhibits an adult appearance (28).

The development of the sympathetic innervation of the pineal gland brings adrenergically regulated processes under neuronal control. For example, prior to innervation it is clear that pineal N-acetyltransferase is adrenergically stimulated by circulating catecholamines (29). When nerves are present and in a resting state, uptake processes remove extracellular catecholamines, maintaining them at substimulatory levels. In the adult this process predominates during the day-time hours, whereas at night, neural stimulation results in the net release of catecholamines. At birth N-acetyltransferase activity does not cycle but is intermediate between adult day and night values during both the day and night. This constant partial activation is a result of tonic stimulation by circulating catecholamines. When innervation of the gland occurs during the first week of life there is a marked decrease in day-time values of N-acetyltransferase, due to uptake, and a marked increase in night-time values, due to release (23,24).

In a recent set of experiments it has been found that the timing of the sharp decrease in day-time N-acetyltransferase activity can be shifted by neonatal hormone treatment (30). Thyroxine treatment accelerates and corticosterone treatment delays this timing. Related studies suggest this is probably due to hormonal-induced changes in the timing of innervation of the gland by sympathetic neurons.

C. Tryptophan→Melatonin Pathway (Figure 2)

The parameters referred to are discussed on a per milligram protein basis. It should be kept in mind that the rat pineal gland weighs about 0.2 mg at birth and about 1 mg at 60 days.

1. Tryptophan hydroxylation: Tryptophan hydroxylation is detectable two days prior to birth. At this time activity is about 50% of adult values and increases about 2-fold during the next four days (28). The capacity of the pineal gland to hydroxylate tryptophan is remarkably high, and exceeds that of all other mammalian tissue, except the raphe-containing midbrain (31). This is a reflection of both high levels of tryptophan hydroxylase and biopterin, its cofactor.

The developmental appearance of biopterin has been recently studied (32). A marked developmental increase occurs during a seven day period starting two days prior to birth. During this period the biopterin/mg protein ratio increases about 8-fold (32).

2. Aromatic amino acid decarboxylase: The activity of this enzyme as measured using dihydroxyphenylalanine as substrate has an unusual developmental pattern (32). For the first three weeks of life values are very low. Significant increases in activity occur at two periods: the end of the third and during the eighth postnatal week. Assuming that a single decarboxylase is responsible for the decarboxylation of both tryptophan and DOPA, it would appear that the development of this step of the tryptophan→melatonin pathway lags behind other components. The two periods of developmental changes have not been related to any other change in pineal or brain function.

As will become obvious after reading the following discussions regarding serotonin (33) and melatonin (34), the low levels of aromatic amino acid decarboxylase early in development do not seem to have a significant influence on the developmental appearance of either serotonin or melatonin, which occur prior to the end of the second week of life. Accordingly, it seems that the activity of this enzyme does not limit serotonin synthesis in the neonatal pineal glands, even though this activity is substantially lower than that seen in the adult.

3. Serotonin: Serotonin in the newborn pineal gland is about 10% of that seen in the adult during daytime (33). Large increases in pineal serotonin occur between days 3 and 11. The day and night values reach adult levels by day 11. Serotonin concentration is a reflection of both synthesis and degradation. Thus, it seems likely that the developmental increase in pineal serotonin concentration reflects both a gradual increase in serotonin production and a gradual decrease in the day-time rate of N-acetylation. The serotonin day/night ratio, which achieves adult proportions during the second week of life, correspondently reflects the day/night difference in the rate of serotonin N-acetylation.

4. Monoamine Oxidase: MAO activity is low at birth, increases markedly between days 13 and 20 and continues to increase at a slow rate after this time (28). There are two types of monoamine oxidase in the pineal gland (35): one associated primarily with parenchymal cells and the other with sympathetic nerves. The developmental appearance of one or the other type has not been studied.

5. Serotonin N-acetyltransferase: N-Acetyltransferase activity is first detectable four days prenatally and gradually increases (23). Day/night differences are not detectable until the 4th day of life. At this time, day values begin to decrease, and night values increase for the next few days, reaching adult levels about the 11th postnatal day. As discussed above, the decrease in enzyme activity likely reflects the ingrowth of nerve endings and their capacity to take up extracellular catecholamines, thus maintaining extracellular levels at substimulatory levels (29). The day/night difference results from neural stimulation of the pineal gland by the SCN. The adrenergic cyclic AMP mechanism which regulates this enzyme is discussed below (see 8).

6. Hydroxyindole-O-methyltransferase: In the rat, the activity of this enzyme is low during the first week after birth, increases sharply during the second week and reaches adult levels by the end of the third week of life (36). This enzyme has been detected in fetal sheep (37) and monkey pineal glands (38) at values which are about 10% of those seen in the adult. The significance of this as it relates to melatonin production is not clear, as the development of the entire pathway in these animals has not been studied. There is a sudden rise in HIOMT activity during the last 4 to 5 days of pregnancy in the ewe, coincident with rapid endocrine changes associated with parturition (37).

7. Melatonin: The developmental appearance of melatonin has been studied in the rat and in two species of hamster (34). In all three the rhythm in melatonin is first detectable during the second week of life and reaches adult concentrations soon after. Light suppression of night-time melatonin values is evident at the end of the second week of life, coinciding with the appearance of the melatonin rhythm.

A recent finding of substantial interest is that the amplitude of the melatonin rhythm is markedly decreased in the aged hamster (34a). The nocturnal increase is barely detectable in either sex. The neural or biochemical changes underlying this have not been identified, and could involve any portion of the melatonin rhythm generating system.

8. Adrenergic-cyclic AMP regulatory system for N-acetyltransferase:

a. Beta-adrenergic receptors: Three to four days prior to birth beta-adrenergic receptors are detectable at less than 5% adult density (39-43). During the next week there is a 14-fold increase in receptor number, with adult levels being achieved during the second week of life. No change in the binding characteristics appears to occur during this period (39,40).

It should be added that as the adult ages, the number of beta-adrenergic receptors in the pineal gland decreases (42). The interesting diurnal difference in receptor number (43), which characterizes the mature pineal gland, has not been studied from a developmental point of view.

b. Adenylate cyclase: The basal activity of adenylate cyclase is at adult levels 3 to 4 days prior to birth (40,44). It is not known when this enzyme develops. Adrenergic stimulation of adenylate cyclase closely follows the increase in beta-adrenergic receptors, with a sharp increase occurring between birth and 4 days of age (44). The distinct difference between the developmental patterns of adenylate cyclase and beta-adrenergic receptors indicates that their development may not be closely linked.

Adenylate cyclase activity can also be stimulated by cholera toxin via a mechanism independent of beta-adrenergic receptors. Cholera toxin activates the enzyme by interacting with a regulatory protein linking the beta-adrenergic receptors and adenylate cyclase. This activation is clearly present 3 days prior to birth and increases substantially during the first three days of life, indicating the regulatory protein is probably present before beta-adrenergic receptors appear.

c. Cyclic AMP stimulation of N-acetyltransferase: Pineal N-acetyltransferase can be stimulated by cholera toxin or dibutyryl cyclic AMP, presumably by increasing the concentration of cyclic AMP or in the case of dibutyryl cyclic AMP, substituting for cyclic AMP (29,40). Stimulation of N-acetyltransferase by these agents is not detectable 3 days prior to birth, indicating that the cyclic AMP sensitive system has not developed at this time. However, there is a sharp increase in responsiveness during the last few days of gestation and the first day of life. By this time the in vitro response on a per mg protein basis is similar to that seen in adult tissue (29,40).

d. Phosphodiesterase activity: Total phosphodiesterase activity in the pineal gland increases gradually during the first week of life and then undergoes an abrupt increase in activity in the day 8 to 16 period (47). Measurements during fetal life have not been reported. An interesting shift in the types of phosphodiesterase present in the pineal gland occurs during

development. At birth there is about a 4:1 ratio of a high K_m form of the enzyme to a low K_m form of the enzyme. By ten weeks this ratio shifts to 1:1, as a result of a greater developmental increase in the low K_m enzyme.

e. Relationship of innervation to development of adrenergic-cyclic AMP regulatory system: It is interesting to note that the adrenergic-cyclic AMP regulatory system controlling N-acetyltransferase activity is nearly mature one day after birth. At this time innervation of the pineal gland is barely detectable. This would lead to the conclusion that the adrenergic-cyclic AMP regulatory system develops independently of innervation, and argues against the attractive hypothesis that neurotransmitters induce the developmental appearance of their receptors.

However, catecholamines available from the circulation might provide a developmental cue. Thus, it would be of interest to examine the developmental appearance of the pineal adrenergic-cyclic AMP regulatory system in animals in which the levels of circulating catecholamines are severely reduced throughout development.

III. Maternally-Derived Melatonin

The available evidence indicates that melatonin is not synthesized in the developing rat prior to the second week of life. However, the conclusion that melatonin is therefore not present in the circulation prior to the second week of life probably is not correct.

Studies in the rat (48), monkey (36), and sheep (49), have shown that [^{3}H]melatonin is transferred from the mother to the fetus via the placental circulation. In addition, it appears that most of the [^{3}H]melatonin in the fetal rat remains in an unmetabolized form (48), and that 10-fold more unmetabolized [^{3}H]melatonin is found in fetal monkey urine as compared to that in the maternal urine (38). In the case of the rat, this is probably a reflection of fetal animals not having the full enzymatic capacity to metabolize melatonin (50). Accordingly, we suspect that melatonin enters the fetus and accumulates, and that the maternal melatonin rhythm is reflected as a fetal melatonin rhythm (38,48). This interesting and important topic requires additional study to determine the actual amounts of melatonin present in the fetal circulation during the day and night.

[^{3}H]Melatonin is also transferred via the milk from the mother to the suckling rat (51). Studies performed using 8-day old rats indicate that these animals have the capacity to metabolize melatonin. This suggests that the melatonin which enters the suckling rat might not accumulate. However, as is true with the question of melatonin in the fetal circulation, further investigation of melatonin in the circulation of the suckling is also required.

The issue of the presence of melatonin in the circulation prior to the development of the capacity to synthesize melatonin is especially important in view of the finding that there is a developmental loss of sensitivity of the rat pituitary gland to melatonin (52). In neonatal rats melatonin inhibits the LHRH-induced release of LH; this is not seen in adults (53).

IV. Comments on the Developmental Schedule of the Melatonin Rhythm Generating System

There are two areas where this schedule (Fig. 4) is of special value. First, it is useful in designing experiments to determine whether development of one component of the system is required for the subsequent development of

another. By selective inhibition of the development of any one component it may be possible to disrupt the development of others. Similarly, it may be possible to identify strains of animals in which one or more components of the system are absent. Such mutants have been especially useful in developmental neurobiology (53).

The second area where this schedule is of use is in survey studies on the effects of drugs, hormones or chemicals on neural development. By monitoring one end point, the pineal melatonin rhythm, investigators can monitor the development of the entire melatonin rhythm generating system. The absence of this rhythm indicates at least one component has not developed. Further study will determine when development was arrested and which components were influenced by the compound of interest.

The melatonin rhythm generating system is one of the few neural regulatory systems which has been extensively described from a developmental approach. It should now provide a basis for studies designed to provide a more thorough understanding of the regulatory mechanisms involved in neural development.

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